REMARKS

Applicants previously canceled Claims 2, 16, 26-35, 40-45, and 47. Applicants have canceled Claims 1, 3-15, 17-25, and 36-39 herein, as being drawn to non-elected inventions or species, and Applicants reserve the right to pursue the subject matter of these claims in one or more continuation or divisional applications as necessary. Applicants have withdrawn Claims 52, 53, 58, and 59, as being drawn to non-elected species of gamma secretase inhibitors. Applicants also have canceled Claim 48, 49, and 54, have amended Claims 46, 50, 51, 55-57, and 60 for clarity herein, and have added new Claims 61-68. Enabling support for the amendments and additions can be found in the application as filed (*See, e.g.*, original claims; Col. 5, paragraph [042]; Col. 7, paragraph [053]; Figure 9; Example 5, paragraph [0169]). Therefore, no new matter is contained in the amendments. Reconsideration of the present application and allowance of pending Claims 46, 48-51, 55-57, and 60 are respectfully requested in view of the amendments and following remarks.

I. Restriction Requirement

The Office Action made final the previous restriction requirement. Accordingly, Applicants have canceled Claims 1, 3-15, 17-25, and 36-39 herein, as being drawn to non-elected inventions. Applicants reserve the right to prosecute the subject matter of the canceled claims in one or more continuation or divisional applications.

In addition, Applicants previously elected DAPT as the species of gamma secretase inhibitor, and human embryonic stem cells as the species of pluripotent cells. Applicants have canceled herein the claims to the extent that they relate to pluripotent cells other than human ES cells. Applicants have withdrawn Claims 52, 53, 58, and 59, as being drawn to non-elected species of gamma secretase inhibitors. As the claims are believed to be in condition for allowance as they relate to the elected species DAPT, Applicants respectfully request that the Patent Office examine the pending claims as they relate to the non-elected species at this time.

II. Objections

A. The Office Action noted that PCT Publication No. WO 02/77204 was cited in the Information Disclosure Statement filed December 27, 2005, but that a copy of that publication was not submitted. Applicants have concurrently filed a Supplemental Information Disclosure Statement, citing and enclosing this publication.

B. The Office Action objected to the specification because it contained embedded hyperlinks and/or other forms of browser-executable code. Applicants have amended the specification herein to delete the objected to phrases, and therefore, this objection should be withdrawn.

C. The Office Action also objected to Claims 51 and 54 for reciting the acronym DAPT, without first identifying the agent or compound. Applicants have canceled Claim 54 and have amended Claims 50 and 51 to recite that DAPT is an acronym for N-[N-(3,5-Difluorophenacetyl-L-alanyl)]-S-phenylglycine t-Butyl Ester. This definition may be found at Col. 5, paragraph [042] of the specification as published, and therefore, no new matter is contained in the amendment. Applicants respectfully request that this objection be withdrawn.

III. Rejection under 35 U.S.C. § 112, first paragraph

Claims 46, 48-51, 54-57, and 60 were rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to comply with the enablement requirement. In particular, the Office Action alleged that the specification is not enabling for the broad genus of distinctly different pluripotent cell populations obtained from an enormous genus of metazoan animals, for the physiological or phenotypical state that is "stabilized," or for the use of a genus of structurally distinct compounds that possess distinctly different biochemical and mechanistic properties that are to be used alone or in combination for the treatment of pluripotent cells. The Office Action alleged that while the specification discloses the passaging of hES cells in an undifferentiated state for more than 40 passages (Example 5), the specification does not disclose the DAPT treatment condition variables (*e.g.*, duration, frequency, or application before or after critical steps) necessary to achieve the reduced number of spontaneously differentiated cells. The Office Action also alleged that the specification does not disclose a nexus between the culture conditions of human ES cells and other pluripotent cell populations known in the art, each of which have their own specific

cell culture requirements. Applicants note that the rejection is moot with respect to Claims 48, 49, and 54, as these claims have been canceled herein. Applicants respectfully submit that the specification is sufficient to enable one of ordinary skill in the art to make and use the currently pending claims without undue experimentation.

The currently pending claims have been amended to recite that the methods involve the maintenance of a human ES cell culture in an undifferentiated state by contacting the cell culture with an inhibitor of the gamma-secretase complex. Similarly, the newly added claims are directed to methods of inhibiting the differentiation of a human ES cell culture, resulting in the increased homogeneity of the cell culture. Support for these amendments and additions may be found, for example, in Example 5, paragraphs [0167]-[0170]. Therefore, the rejection is moot with respect to pluripotent cells other than human ES cells. The claims as amended also clarify that the cells are being maintained in an undifferentiated state. Therefore, the rejection is also moot with respect to the physiological or phenotypical state that is being stabilized or maintained.

The Office Action acknowledged that the specification discloses the passaging of hES cells in an undifferentiated state for several passages in Example 5, but the Office Action alleged that the specification does not disclose the DAPT treatment condition variables necessary to achieve the reduced number of spontaneously differentiated cells. Applicants respectfully submit that the specification does provide sufficient guidance for one skilled in the art to practice the claimed invention without exercising undue experimentation. For example, the specification provides that hES cell cultures treated with DAPT at a concentration of 50 μM (after trypsin passaging and SSEA4 sorting), as compared to untreated cultures, "express high levels of SSEA4 and Notch1 (67.8%; Fr. A), which is indicative of the undifferentiated state" of the cells (Example 5, Col. 22, paragraphs [0167] - [0168]; and Figs. 9 and 10). Therefore, the Example shows that inhibition of gamma secretase by adding DAPT after trypsin passaging and SSEA4 sorting maintains the hES cells in an undifferentiated state and/or inhibits hES cell differentiation. One of ordinary skill in the art would be able to practice the claimed invention without undue experimentation based on the present disclosure.

In addition, the Office Action identified two references regarding the use of DAPT in pluripotent stem cell cultures. The Office Action alleged that Crawford *et al.* teach that 1 μM DAPT enhances the neuronal differentiation of ES cell-derived embryoid bodies, which comprise pluripotent cell populations. Applicants respectfully submit that Crawford *et al.* describe the effect of DAPT on mouse ES cells, rather than human ES cells. In addition, Crawford *et al.* use DAPT in combination with retinoic acid, which is known in the art to cause the neurodifferentiation of ES cells. Similarly, the Office Action alleged that Gal *et al.* expose GMB cells to DAPT treatment, disrupting the formation of neurospheres, indicating a loss of stem-cell like properties such as self-renewal. Again, Gal *et al.* also do not use human ES cells. For at least these reasons, Crawford *et al.* and Gal *et al.* are not relevant to the enablement of the present invention relating to methods for maintaining human ES cell cultures in an undifferentiated state by treatment of the cultures with DAPT.

The specification is sufficient to enable one of ordinary skill in the art to make and use the claimed invention without exercising undue experimentation. Therefore, the rejection under 35 U.S.C. § 112, first paragraph with respect to the enablement requirement should be withdrawn.

IV. Rejections under 35 U.S.C. § 112, second paragraph

Claims 46, 48-51, 54-57, and 60 were rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. In particular, the Office Action alleged that the claims were unclear based on the recitation of certain terms or phrases. The rejection is moot with respect to Claims 48, 48, and 54, as these claims have been canceled herein. Applicants respectfully submit that the currently pending claims particularly point out and distinctly claim the subject matter of the invention.

Claim 46 and dependent Claims 48-51, 54-57, and 60 were rejected as being unclear in the recitation of the term "stabilize." The Office Action alleged that "stabilize" is a relative term and that the specification does not provide a standard for ascertaining the requisite degree, such as a reference value or phenotype to clearly distinguish a "stabilized" cell versus "non-stabilized"

cells. In addition, the Office Action alleged that the specification teaches that the cell culture includes some ES cells that are undergoing differentiation into pluripotent progenitor cells of different cell types, and therefore, that it is unclear which pluripotent cell type is being stabilized and what phenotype that cell has. Applicants respectfully submit that this rejection is moot with respect to the currently pending claims, which have been amended to recite that the methods involve the maintenance of a human ES cell culture in an undifferentiated state, and with respect to the newly added claims which are directed to methods of inhibiting the differentiation of a human ES cell culture. Support for these amendments and additions may be found, for example, in Example 5, paragraphs [0167]-[0170]. The undifferentiated state of the cell culture may be determined, for example, by detecting expression of certain pluripotency markers (*e.g.*, SSEA4 and Notch1), by detecting a lack of expression of differentiation markers (*e.g.*, HNF4alpha and GATA-4), and by examining cell morphology, etc. as is well known in the art. See, for example, Example 5 and Col. 5, paragraph [0043]. Therefore, Applicants respectfully request that this rejection be withdrawn.

Claim 50 and dependent Claims 51 and 54 were rejected as being indefinite in the recitation of the term "non-transition state analogue." Applicants have canceled Claim 54, and have deleted the objected to phrase from Claim 50. Therefore, this rejection is moot.

Applicants respectfully submit that the currently pending claims particularly point out and distinctly claim the subject matter of the invention. Therefore, the rejections under 35 U.S.C. § 112, second paragraph should be withdrawn.

V. Rejections under 35 U.S.C. § 102

Claims 46 and 60 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Karanu *et al.* (2000, J. Exp. Med. 192(9):1365-72), as evidenced by Small *et al.* (2001, J. Biol. Chem. 276(34):32022-30). In particular, the Office Action asserted that Karanu *et al.* teach a method of maintaining the survival and expansion of human hematopoetic stem cells in cell culture, the method comprising the administration of soluble human Jagged-1 extracellular domain, which is recognized in the art as an inhibitor of Notch signaling. Applicants respectfully submit that the currently pending claims are novel over the teachings of Karanu *et al.*

A claim is anticipated only when a single prior art reference expressly or inherently teaches each and every feature of the claim. *See Verdegaal Bros. v. Union Oil Co. of Cal.*, 814 F.2d 628 (Fed. Cir. 1987). Karanu *et al.* do not teach each and every feature of the presently claimed invention. The currently pending claims are directed to methods of maintaining a human embryonic stem cell culture in an undifferentiated state by contacting the cell culture with an inhibitor of the gamma-secretase complex. Karanu *et al.* teach the survival and expansion of human hematopoetic stem cells in cell culture by administering a soluble human Jagged-1 extracellular domain. Karanu *et al.* do not teach or suggest the use of the soluble human Jagged-1 extracellular domain for the maintenance of human ES cells in an undifferentiated state. Moreover, while the soluble human Jagged-1 extracellular domain is an inhibitor of Notch signaling, there is no teaching or suggestion by Karanu *et al.* that the soluble human Jagged-1 extracellular domain is an inhibitor of the gamma-secretase complex as is required by the claims. As is noted in the background of the present application, the gamma secretase complex can signal through a number of signaling pathways, in addition to signaling through the Notch pathway. *See* Col. 2, para. [0014].

Karanu *et al.* do not teach each and every feature of the claimed methods of the present invention. Therefore, the rejection under 35 U.S.C. § 102(b) should be withdrawn.

VI. Rejections under 35 U.S.C. § 103

A. Claims 46, 48-49, and 60 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Karanu *et al.* (2000, J. Exp. Med 192(9):1365-72), as evidenced by Small *et al.* (2001, J. Biol. Chem. 276(34):32022-30) and Walsh *et al.* (2003, Acta Pathological, Microbiolica et Immunologica Scandinavica (APMIS) 111(1):197-210). Applicants respectfully submit that the presently claimed invention is not obvious over Karanu *et al.*, in view of Small *et al.* and Walsh *et al.*

In particular, the Office Action asserted that Karanu *et al.* teach a method of maintaining the survival and expansion of human hematopoetic stem cells in cell culture, the method comprising the administration of soluble human Jagged-1 extracellular domain, which is recognized in the art as an inhibitor of Notch signaling. The Office Action further alleged that

Karanu *et al.* teach that the activity of that molecule provides an opportunity for the optimization for clinical protocols aimed at ex vivo expansion and gene transfer of human stem cells, but that Karanu *et al.* do not teach the cell culture to be a human ES cell culture. The Office Action alleged that Walsh *et al.* teach that Notch molecules are expressed in human ES cells and that Notch signaling is important for maintaining ES cells in an undifferentiated state and for directing their differentiation. Accordingly, the Office Action concluded that it would have been obvious to try administering the soluble human Jagged-1 extracellular domain to human ES cells to maintain the survival and expansion of the cells in an undifferentiated state.

Applicants respectfully submit that there are numerous fundamental differences between hematopoietic stem cells and embryonic stem cells. Accordingly, it is extremely unpredictable whether the relationship between inhibiting Notch signaling and the maintenance and expansion of hematopoietic stem cells as shown by Karanu *et al.* would translate to ES cells. Moreover, as discussed above, there is no teaching or suggestion by Karanu *et al.* that the soluble human Jagged-1 extracellular domain is an inhibitor of the gamma-secretase complex as is required by the claims. Walsh *et al.* do not cure these deficiencies. In fact, Walsh *et al.* teach away from the claimed invention, because Walsh *et al.* describe that activating Notch signaling enhances ES cell self-renewal, contrary to the teaching of the present specification, which shows that inhibiting gamma secretase (which inhibits notch signaling, among other things) enhances ES cell self renewal. Hence, Walsh *et al.* teach away from the claimed invention.

Karanu *et al.* and Walsh *et al.* do not teach or suggest the presently claimed methods. Therefore, the rejection under 35 U.S.C. § 103(a) should be withdrawn.

B. Claims 46, 50-51, 54, and 55-57 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Karanu *et al.* (J. Exp. Med 192(9):1365-1372, 2000), as evidenced by Small *et al.* (J. Biol. Chem. 276(34):32022-32030, 2001) and Walsh *et al.* (Acta Pathological, Microbiolica et Immunologica Scandinavica (APMIS) 111(1):197-210, 2003), as applied to claims 46, 48-49, and 60 above, and in further view of Dovey *et al.* (J. Neurochem. 76:173-181, 2001; * of record in IDS), as evidenced by Pera *et al.* (J. Cell Science 113:5-10, 2000) and Nakhei *et al.* (Nucleic Acids Res. 26(2):497-504, 1998). In particular, the Office Action asserted that Karanu *et al.* do not teach the use of the gamma secretase inhibitor DAPT to maintain and

expand stem cells in an undifferentiated state. The Office Action alleged that Dovey *et al.* taught that DAPT is an inhibitor of gamma secretase and would likely negatively affect the cleavage of other gamma secretase targets, such as Notch. The Office Action noted that Dovey *et al.* do not teach the limitations of Claims 55-57 (maintenance for at least 10 passages, 60% cells expressing SSEA4 and Notch1, and less than 20% cells expressing HNF4alpha, respectively), however, the Office Action asserted that those limitations are necessarily inherent features of an ES cell passaged in the presence of an inhibitor of Notch signaling. Accordingly, the Office Action concluded that it would have been obvious to substitute the soluble human Jagged-1 extracellular domain as taught by Karanu *et al.* with DAPT as taught by Dovey *et al.* in order to maintain ES cells in an undifferentiated state. The Office Action asserted that such substitution would have a reasonable chance of success because it was a simple substitution of one inhibitor of Notch signaling for another. Applicants respectfully submit that the presently claimed invention is not obvious over the cited references.

As discussed above, Karanu *et al.* teach the use of a soluble human Jagged-1 extracellular domain to maintain and expand hematopoietic stem cells in culture. Applicants respectfully submit that there are numerous fundamental differences between hematopoietic stem cells and embryonic stem cells. Accordingly, it is extremely unpredictable whether the relationship between inhibiting Notch signaling in hematopoietic stem cells as shown by Karanu *et al.* would translate to ES cells. Dovey *et al.* and the other cited references do not cure this deficiency.

The cited references do not teach or suggest the presently claimed methods. Therefore, the rejection under 35 U.S.C. § 103(a) should be withdrawn.

CONCLUSION

Applicants believe that the present application, as amended, is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. The foregoing is submitted as a full and complete response to the Office Action mailed October 3, 2007.

A petition for a two-month extension of time, as well as the required fee therefore, are enclosed herewith. No additional fees are believed due at this time. However, please charge any

fees that may be due, or credit any overpayment, to Deposit Account 19-5029 (Ref. No.: 18377-0063). In addition, if there are any issues that can be resolved by a telephone conference or an Examiner's amendment, the Examiner is invited and encouraged to call the undersigned attorney at (404) 853-8000.

Respectfully submitted,

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